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Research paper

Characterization of active compounds from Jatropha gossypifolia L. based on HPTLC

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Abstract

Jatropha gossypifolia L. is one of plants that come from Euphorbiaceae family.That is used to be a medicine, particularly as an anticancer. The previous research has reported that the root extract of J.gossypifolia L. (EEAJG) has an ability to kill the cervix cancer cells and breast cancer cells. The cytotoxicity of EEAJG on breast cancer cell lines (T47D) and cervix cancer cell lines (Hella) respectively contained by 43.57 mg/mL and 4.32 mg/mL. The consistency of active compouds on traditional medicine can assurance the biological activity of medicinal plants. Fingerprint profiles of active compouds is either one that can be used to indicate the composition of secondary metabolites in medicinal plants. In this study aims to obtain a fingerprint profiles of active compouds in EEAJG to ensure biological activity as an anticancer based on HPTLC.
The root extract of J.gossypifolia L. obtained from extraction by maceration with ethanol. Fingerprint profiles of active compouds can be identified by using High Performance Thin Layer Chromatography (HPLC). Secondary metabolites of EEAJG separated by TLC techniques and scaned wavelength of 254 nm and 366 nm with CAMAG TLC scanner 3. The chromatogram were recorded. The results showed that the chemical compounds were detected at 254 nm produced ten peaks with maximum absorbance at 250-350 nm region. The chemical compounds that were detected at 366 nm produced six peaks with absorbance maximum at 200-350 nm region.

Keywords: *Fingerprint, active compounds, anticancer, ethanolic extract of Jatropha gossypifolia L.*

1. Introduction

According to WHO (2011), the raw material of natural medicine products can be part of the plants, that is leaves, flowers, fruits, seeds, stems, roots or whole plant parts that have not undergone a process or has undergone a process of simple processing. It can also be extracts or fractions from the process of extraction, concentration and separation. Generally in the plants, the chemical compounds are responsible for generating therapeutic activity involving multicomponent well as a multitude of chemical compounds content. Therefore to ensure the correctness and regularity of natural material products need to be standardized (Bauer, 1998; Busse, 2000; Yadav and Dixit, 2008). Based on the decision of National Agency of Drug and Food Control (BPOM) of the Basic Provisions classification and marking of Natural Drugs Indonesia, the dosage of natural ingredients meets the criteria: safe, efficacy has been proven scientifically with preclinical trials or clinical trials, have been conducted standardization of raw materials used in the finished dosage, and meets the quality requirements applicable (BPOM, 2004).
In natural medicine products, there are chemical compounds responsible for the activity. Chemical compounds with known therapeutic activity is a material or group of materials that are chemically defined and has been known to give a therapeutic activity. Therefore the quality and safety of medicinal products of natural materials are determined by the composition and concentration of chemical compounds contained in preparations (Bauer, 1998; Busse, 2000; WHO, 2005; Bandaranayake, 2006). Fingerprint characteristics of chemical compounds that is semi-quantitative of the extract or active fraction obtained can be analyzed using chromatographic techniques to document quality, and therefore could provide information for the consistency of each production (Bauer, 1998; Busse, 2000).
Jatropha gossypifolia L. which is known as "jarak cina", is one of the plants of the genus Jatropha with family Euphorbiaceae (Hutpea, 1994). Biological activity has been reported as antibacterial (Rajani et al, 2006; Ogundare, 2007), cytotoxic against cancer cells (Kupchan et al, 1970, Rofida et al, 2015), antiprotozoal (Jansen et al, 2010; Gbeassor et al, 1989), anticoagulant (Oduola et al, 2005), antiinflammatory (Panda et al, 2009), hepatoprotective (Panda et al, 2009), insecticidal (Valencia et al, 2006), inhibition of AChE (Sigh and Singh, 2002).
The result of biological activity screening in organ leaves, bark, fruit and roots J.gossypifolia L. showed that the ethanol extract of the roots J.gossypifolia L has potential as an anticancer on breast and cervical cancer. The previous research has reported that the root extract J.gossypifolia L. (EEAJG) has an ability to kill the cells of the cervix and breast cells. The cytotoxicity of EEAJG on Cell Lines T47D and Hella each contained at 43.57 mg/mL and 4.32 mg/mL. To ensure the consistency of biological activity has been previously reported, it is necessary to semi-quantitative data concerning the characteristics of the fingerprint compounds J.gossypifolia root extract.

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2. Methods

2.1. Materials and equipment

The root extract of J.gossypifolia L. (EEAJG), n-hexan (pro analysis), ethyl acetat (pro analysis), chloroform (pro analysis), TLC Plate silica gel F254 (Merck), CAMAG TLC Scaner 3, Ferri Chloride (Merck), anisaldehyde-sulfuric acid , NH4OH(p) (Merck), Chamber CAMAG.

2.2. Phytochemical identification

Identification of chemical compounds in EEAJG using TLC technique. EEACG were performed on silica gel F254, 5 µL of EEAJG were applied on the plate 5x10 cm. The plate of silica gel F254 developed in Chamber CAMAG that has been saturated by mobile phase a mixture of hexane: chloroform: ethyl acetate = 5: 3: 3. The plate developed throughout eight cm than air dried. Chemical compounds have been separated, documented on observations at UV 254 nm and 366 nm. Subsequently each plate silica sprayed with reagent ammonia fumes, anisaldehyde-sulfuric acid, and FeCl3 to detected flavonoids, terpenoids, and polyphenols compounds. Chemical compounds were documented on direct observations and at UV 366 nm.

2.3. HPTLC analysis

Fingerprint profile of EEAJG analyzed using HPTLC techniques. EEACG were performed on silica gel F254, aliquot 5 mL of EEAJG were applied on the plate 5x10 cm. Plate of silica gel F254 developed in Chamber CAMAG that has been saturated by mobile phase a mixture of hexane: chloroform: ethyl acetate = 5: 3: 3. Plat developed throughout eight cm, air dried and scanned at wavelength of 254 nm and 366 nm using CAMAG TLC Scanner 3. Profile chromatograms produced were recorded. Against the separated compounds, respectively scanned at wavelength of 200-700 nm to determine the spectrum and maximum absorption of the compounds. Spectrum profile were recorded.

3. Results and Discussion

Phytochemical of EEAJG identified by TLC techniques and than derivatized by ammonia fumes, anisaldehyd-sulfuric acid, and FeCl3 reagents for showing of flavonoids, terpenoida, and polyphenols compounds. Chromatogram profile of EEAJG shown in figure 1.

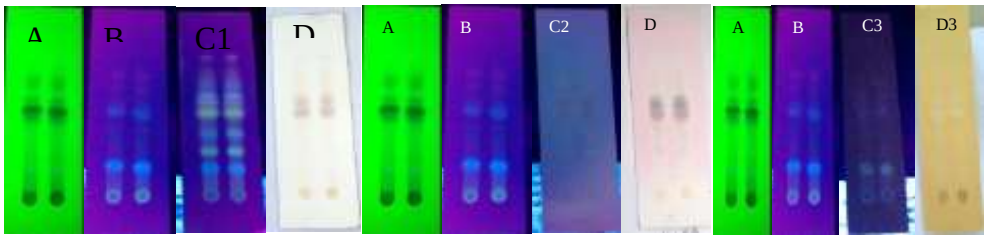


Fig 1: TLC profile of EEAJG, stationary phase: silica gel F254; mobile phase: hexan:chloroform:aethyl acetat=5:3:3; [A. UV 254; B. UV 366; C. Derivatized, UV 366 (C1. ammonia fumes, C2. anisaldehyd-sulfuric acid

reagent, C3. FeCl3 reagent); D. Derivatized, Visual (D1. ammonia fumes, D2. anisaldehyd-sulfuric acid reagent, D3. FeCl3 reagent)].

According Debenedetti (2009), chromatographic technique can be used for analysis, identification, purification and quantification of active chemical compounds in plants. TLC is one of a chromatographic technique that can be used to identify active chemical compounds in plants. The mixture of chemical compounds separated by adsorption mechanism. These chemical compounds are separated by polarity when the compound moves with the mobile phase and interact with the surface of the stationary phase. Separate chemical compounds that can be observed directly, under ultraviolet light or by spraying with a chemical reagent.

Identification of active chemical compounds on EEAJG using TLC technique, showed that there were from the class of flavonoids, terpenoids and phenolic compounds. Based on Figure 1, derivate flavonoids and terpenoids on EEAJG shown by the spot stains, which can reduce the fluorescence on silica plate at UV 254 nm. Derived flavonoids compounds, at UV 366 nm indicate the presence of fluorescence in blue and purplish red colour (Mabry et al, 1970; Harborne, 1984; Spanberg 2008; Debenedetti, 2009). After derivatized with ammonia vapour, flavonoids compound shown by the increasingly strong blue fluorescence. According Mabry et al (1970), these compounds were isoflavones. In addition, there are also changes colour to yellowish green after derivatisation with ammonia vapour. The flavonoids were flavones and flavanones lacking a free 5-OH (Mabry et al, 1970). While the terpenoid compounds do not give a specific colour at UV 366 nm. Terpenoid compounds can also be highlighted in red to purplish after sprayed with an anisaldehyde-sulfuric acid reagent. While in the class of phenolic compounds is shown in black after a given exposure ferric chloride reagent (Harborne, 1984; Spanberg 2008; Debenedetti, 2009).

Fingerprint characteristics in qualitative and semi-quantitative of chemical compounds contained in EEAJG analysed using High-Performance Thin Layer Chromatography (HPTLC). Detection of active chemical compounds performed at two wavelengths that is UV 254 nm and 366 nm. From the analysis of HPTLC, obtained the fingerprint of EEAJG which indicates there were 10 peaks and 6 peaks when be detected at the wavelength of 254 nm and 366 nm. Profile chromatograms obtained from scanning at the wavelength of 254 nm and 365 nm shown in Figure 2.

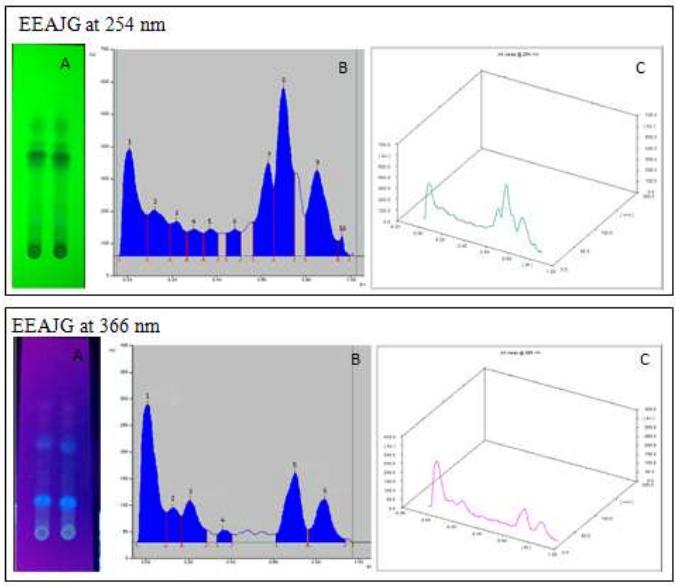


Fig 2: Chromatogram profile of EEAJG analysed by HPTLC at wavelength 254 nm and 366 nm (A) TLC profile, (B) HPTLC profile (2D), (C) HPTLC profile (3D).

From the results of scanning at a wavelength of 254 nm and 366 nm, can be calculated retention factor value and the area of active chemical compounds that show qualitative and semiquantitative presence. Retention factor value and the area each one of active chemical compounds shown in Table 1.

Table 1: Retention Factor Value and Area Chemical Compounds of EEAJG

Peak	Retention factor		Area (%)	
	254 nm	366 nm	254 nm	366 nm
1	0,04	0,04	18,18%	40,12%
2	0,09	0,10	8,96%	8,36%
3	0,19	0,17	4,90%	12,48%
4	0,27	-	4,01%	-
5	0,34	0,34	3,63%	2,88%
6	0,44	-	3,62%	-
7	0,56	-	13,19%	-
8	0,66	0,61	25,27%	21,73%
9	0,80	0,77	17,14%	14,44%
10	0,95	-	1,13%	-

Chemical compounds were detected at a wavelength of 254 nm and 365, was scanned at a wavelength of 200-700 nm to obtain spectrum data and wavelength of maximum absorption of chemical compounds on EEAJG. The analysis showed that the chemical compounds were detected at 254 nm, giving a maximum absorption at a wavelength in the 250-350 nm, by 2-3 peaks. While the analysis of chemical compounds was detected at 366 nm, giving a maximum absorption at a wavelength in the region of 200-350 nm, by 2-3 peaks. Spectrum of chemical compounds on EEAJG shown in Figure 3.

Based on spectrum of active chemical compounds on EEAJG, can be obtained a wavelength of absorption of the greatest of these chemical compounds. The maximum wavelength can be used as a quantitative and qualitative identification of a compound (Srivastava, 2011). The maximum wavelength of chemical compounds on EEAJG shown Table 2.

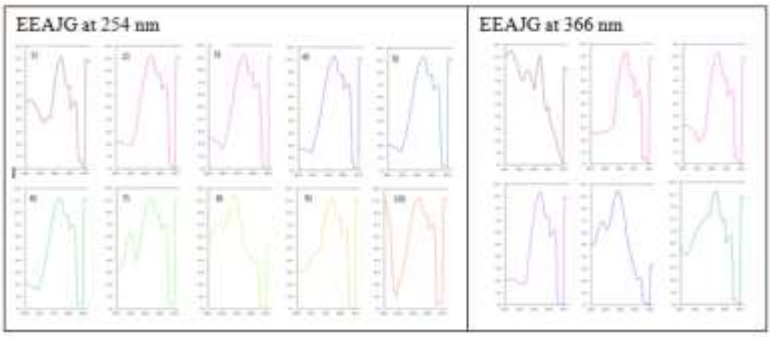


Fig 3: Spectrum of chemical compounds EEAJG were detected at 254 nm and 366 nm.

Table 2: The Maximum Wavelength Chemical Compounds of EEAJG

Peak	Retention factor		λmax chemical compounds of EEAJG	
	254 nm	366 nm	Detected at 254 nm	Detected at 366 nm
1	0,04	0,04	320 nm	320 nm
2	0,09	0,10	320 nm	325 nm
3	0,19	0,17	315 nm	325 nm

4	0,27	-	315 nm	-
5	0,34	0,34	320 nm	320 nm
6	0,44	-	315 nm	-
7	0,56	-	320 nm	-
8	0,66	0,61	285 nm	295 nm
9	0,80	0,77	320 nm	-
10	0,95	-	320 nm	320 nm

According Harborne (1984), the flavones compounds provide maximum absorption in the wavelength range 330-350 nm. While the flavanones compounds provide maximum absorption at 275-290 nm region. Isoflavone compounds provide maximum absorption at 250-325 nm region.

4. Conclusion

The results showed that the active chemical compounds were detected at 254 nm produced ten peaks with maximum absorbance at 250-350 nm region. The active chemical compounds that were detected at 366 nm produced six peaks with absorbance maximum at 200-350 nm region.

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